

Examining the Effects of Serotonin on Medium Spiny Neurons (MSNs)

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Background

- Serotonin is a key hormone for our feelings of happiness and is also associated with depression.
- Pregnant woman with chronic depression are prescribed selective serotonin reuptake inhibitors (SSRIs).
- The effects of SSRIs on the developing embryo, especially on its developing Medium Spiny Neurons (MSNs), are under examined.
- This knowledge might better inform us about the safety regarding the consumption of SSRIs by pregnant women.

HYPOTHESIS: Serotonin will affect the development of MSNs.

Methods

1. Dissect embryonic mouse brain (diagram)
2. Allow MSNs to grow 19 div
3. Fix
4. Prepare coverslips by incubating overnight with immunofluorescent primary antibody DARPP-32, a protein expressed in MSNs
5. Visualize with epifluorescent microscope at 20x objective
6. Quantify dendritic arborization using Sholl Analysis

References

Mayo Clinic (2019). The most commonly prescribed type of antidepressant. Retrieved July 03, 2020, from <https://www.mayoclinic.org/diseases-conditions/depression/in-depth/ssris/art-20044825>
Mondal, S. K. (2017). Manual of Histological Techniques (1st ed.). Jaypee Brothers Medical Publishers Pvt Ltd. https://doi.org/10.5005/jp/books/13001_20
Penrod, R. D., Campagna, J., Panneck, T., Preese, L., & Lanier, L. M. (2015). The presence of cortical neurons in striatal-cortical co-cultures alters the effects of dopamine and BDNF on medium spiny neuron dendritic development. *Frontiers in cellular neuroscience*, 9, 269. <https://doi.org/10.3389/fncel.2015.00269>

Figure 1:

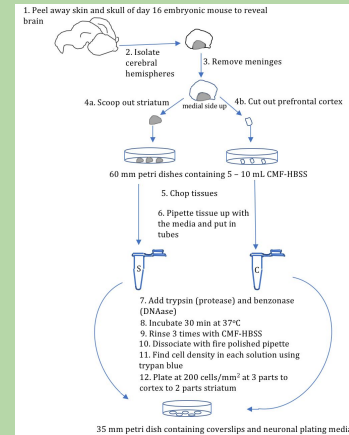
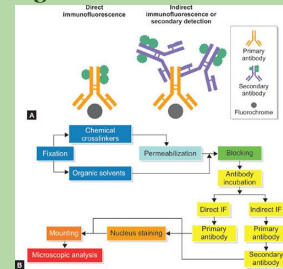


Figure 2:

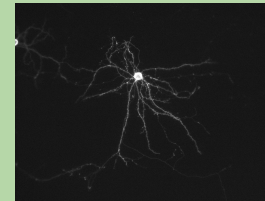


The steps followed in order to prepare the neuronal cell cultures are shown in Figure 1.

Once these cultures are prepared, they are subjected to treatment with primary and secondary antibodies. The process is shown in Figure 2.

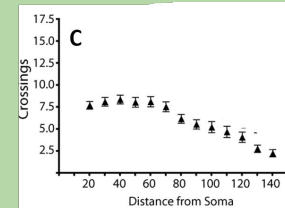
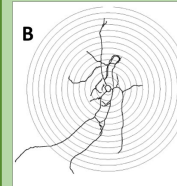
The cells are treated with primary antibodies in order to detect the primary antigen in the cell culture. The secondary antibodies have fluorophores attached to them, which aid in the visualization of these neurons, as shown in Figure 3. This image is then traced - a step required for Sholl Analysis.

Figure 3:



Analysis

Sholl analysis: - Concentric circles radiating from soma are used to evaluate the complexity of the dendritic arbor. The number of crossings of each dendrite at a certain distance from the soma is the plotted, as shown below.



Conclusion and Future Directions

The increase or decrease in the number of crossings would indicate if serotonin leads to increase or decrease in the density of the dendritic arbor of MSNs. In the future, the co-application of dopamine and serotonin can be performed in order to observe if serotonin and dopamine act synergistically or antagonistically.